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BOTANICAL GAZETTE

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ON THE NATURE OF THE STIMULUS WHICH CAUSES THE CHANGE OF FORM IN POLYMORPHIC GREEN ALGÆ.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY XXII.

(WITH PLATES XVII AND XVIII)

BURTON EDWARD LIVINGSTON.

Introductory.

ALTHOUGH the work of Klebs and others[1] has demonstrated clearly that there exist a number of polymorphic species among the green algæ, yet up to the present time the exact nature of the stimulus which brings about the change in the manner of growth has not been determined. To throw some light upon this question the experiments to be described in the present paper were begun. They were carried on in part at the Botanical Laboratory of the University of Michigan, during the spring and summer of 1898, and in greater part at the Hull Botanical Laboratory of the University of Chicago, during the year just past.

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[1] FAMINTZIN, A.: Die anorganische Salze als Hilfsmittel zum Studium der Entwickelung niederer chlorophyllhaltigen Organismen. Mélanges biol. St. Petersbourg **8**: 225. 1871.

CIENKOWSKY, L.: 1. Zur Morphologie der Ulothricheen. Mélanges biol. St. Petersbourg **9**: 531. 1876.

——— : 2. Ueber Palmellenzustand bei Stigeoclonium. Bot. Zeit. **34**: 17. 1876.

GAY, F.: Recherches sur le développement et la classification de quelques algues vertes. Paris, 1891.

KLEBS, G.: Die Bedingungen der Fortpflanzung bei einigen Algen und Pilzen. Jena, 1896.

Descriptive.

A species of Stigeoclonium (perhaps a form of *S. tenue*), found growing with Pleurococcus, etc., on moist bark at Ann Arbor, was chosen for experimentation. It shows two very distinct and easily distinguishable forms according to the culture medium in which it is grown. The normal form is that of Palmella. A culture of this form shows the surface of the fluid covered with spherical cells 12 to 15 μ in diameter. The cells multiply by division in planes generally vertical and at right angles to each other, and the daughter cells separate more or less completely after division is accomplished (*figs. 1, 6, 12, 27*). The walls are quite thick and somewhat gelatinous on the exterior, though not by any means so markedly as in the form studied by Cienkowsky (*l. c., 2*), and the protoplasm is quite coarsely granular. The chloroplast has the form of a hollow spherical shell with an opening in one side, which has a diameter about equal to the sphere's radius, varying somewhat in different cells. Sometimes the opening is nearly a great circle of the sphere (*fig. 1, a, b*). This opening in the chloroplast is probably the "bright spot" described by Cienkowsky. There is always a pyrenoid present in each cell, and sometimes two are found. This body has a diameter of about 3 μ, and lies in a thickened region in the chloroplast. When the cell is about to divide the chloroplast separates into two parts, each part taking a half of the original pyrenoid, and a wall forms between the two portions (*fig. 1, c*). The plane of division always intersects the plane of the opening in the chloroplast. Then the two hemispherical cells become more and more spherical, splitting the wall between them along its middle lamella, until finally, if nothing prevents, they come to lie as two separate spheres side by side (*fig. 1, a*). Very often division proceeds more rapidly than this rounding off, and there results a group of four or more cells making contact with each other in plane surfaces (*fig. 6*). Often this process is continued without any parting till there results a plate of cells spread out over the surface of the nutrient medium. Sometimes, too, the planes of division lie parallel to the surface of the medium as well as perpendicular to it, and a parenchyma-like mass of polygonal cells is formed, projecting down into the fluid and up into the air (*figs. 6, 24*). After division the chloroplast enlarges until it occupies about as much of the daughter cell as the mother chloroplast did of the mother cell. The form just described will be termed throughout this paper the *palmella form.*

In the other form we have a very different mode of growth. The cells are not spherical, but cylindrical; they divide, excepting at the origin of a branch, only by transverse planes, and show no tendency to break apart, but remain closely connected to form branching filaments (*figs. 2, 8, 18*). This will be termed in the following discussion the *filamentous form.* The cells of the filaments are 4.5 to 10μ in diameter and from two to four times as long. The longest filaments are 175 to 200μ long and their branches extend in all directions. No true hairs have been seen, but often the tips of the filaments are very narrow. The protoplasm is not granular, as in the palmella form, but usually contains two to seven refractive bodies smaller than the pyrenoid, but looking much like it. They are probably of an oily nature. They have a diameter of about 1.5 μ, that of the pyrenoid being, as in the other form, about 3 μ. The chloroplast is elongated to suit the cell and becomes somewhat trough-shaped; lying along one side of the cylindrical wall it reaches about one half way around the cell and usually partially covers one end, sometimes both. Its opening is thus longer than in the other form. The pyrenoid lies somewhere in a thickened part of the chloroplast.

Reproduction in both forms is accomplished by comparatively large asexual biciliate zoospores. These are the microzoospores of Klebs. I have observed no conjugating zoospores. The chloroplast of the mother cell divides into four to eight parts along curiously parallel planes running nearly at right angles to the plane of the opening (*fig. 1*). Each of these portions, with its share of the protoplasm, becomes a zoospore. After division is complete and the zoospores are nearly formed, the daughter chloroplasts often lose much of their bright green color and become pale. Palmelloid cells usually produce seven or eight zoospores; the number produced by a filament cell depends upon its size. The zoospores are ellipsoidal to spherical when discharged into the water by the rupture of the parent wall, and immediately begin to move about, swimming by means of two long cilia (*fig. 3*). Each contains a pyrenoid, and often several smaller granules; the chloroplast can be seen lying against the wall opposite the origin of the cilia and extending in its usual cup-shaped form somewhat more than half way around the cell. The dimensions of motile zoospores are from $3\times 6\mu$ to $6\times 9\mu$. After swimming freely for several hours, the motion of the cilia becomes sluggish and finally ceases altogether; the zoospore comes to rest and assumes the spherical form. The diameter is now not far from 6μ. The chloroplast then regains its original bright color, the cell enlarges, and, if conditions are favorable, may go over imperceptibly into the palmella form. The cell thus produced may divide a number of times, as described above, and then produce more zoospores; or it may produce them immediately upon the attainment of its full size, or even earlier; or it may become a resting cell and remain quiescent indefinitely.[2] However, a zoospore does not usually show this mode of growth. It generally elongates as it lies on the surface (or sometimes at the bottom) of the fluid, and becomes a cylinder with rounded ends. As it grows longer it bends so that it becomes somewhat crescent-shaped, semicircular, or even horseshoe shaped, and soon divides into two cells by a transverse wall near its middle (*figs. 8, 32*). Thus a filament is started, which soon branches. Its cells produce zoospores sooner or later, according to conditions.

Methods.

Pure cultures were first obtained by growing in nutrient solution in suspended drops; then the material was transferred to small, loosely covered glass dishes containing from 5^{cc} to 10^{cc} of solution. The cultures stood on glass shelves close against the panes of an east or west window, and were always shaded from the direct rays of the sun by a curtain of white muslin, which was left constantly in position during the high temperatures of the summer months. Examination of cultures was made from time to time by transferring the dish with its contents directly to the stage of the microscope and using an objective of medium low power.

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[2] KLEBS, G.: Loc. cit.

PRINGSHEIM, N.: Ueber die Dauerschwärmer des Wassernetzes. Monatssch. d. Berliner Akad. 1860.

The culture media used were modifications of the well-known fluid of Knop,[3] consisting of the following salts: calcium nitrate, four parts; magnesium sulfate, one part; potassium nitrate, one part; di-potassium acid phosphate, one part; iron, a trace. Owing to the extreme weakness of the solutions to be used, it was deemed advisable to secure at the outset, as nearly as might be, the exact proportions of the constituent salts here given, and to this end the following method was adopted. By reference to tables of the physical properties of solutions,[4] the corresponding specific gravity and gram-molecular strength of solutions of the first three of the above salts were found. By the use of these data a stock solution of each of these compounds was made up, so that it contained a specific number of gram-molecules per liter. The specific gravity bottle was used in these determinations, and temperature was always taken into account. For the fourth salt in the list data could not be found; therefore it was formed in solution from normal solutions of phosphoric acid and potassium hydroxid. The physical constants for these could be found. The accuracy of this method for producing K_2HPO_4 was tested by volumetric analysis and found to be satisfactory. The specific gravity of a normal solution of this salt so made was found to be 1.13207 at 15° C. Redistilled water was used throughout all of the work.

The stock solutions thus prepared were kept in flasks tightly stoppered with rubber; their specific gravity was taken from time to time, and water was added as it was lost by evaporation. Knowing the gram-molecular, and hence the percentage, strength of these stock solutions, any strength of Knop's solution may readily be made up from them; and solutions so made up are much more accurate in their proportions than those made in the ordinary way; for in dealing with salts such as magnesium sulfate and calcium nitrate the amount of water contained in the compound as taken from the laboratory bottle is always an uncertain quantity. All troubles arising from crystalline and amorphous, as well as deliquescing, salts may thus be readily avoided. The exact amount of ferric salts in my solution was not determined. The transfers of material from an old culture to a new were made, after a pure culture was obtained, by means of a needle. This was first heated in a flame and then cooled by plunging in the new culture fluid. Bits of steel always scaled off in this operation, and these furnished sufficient iron for the plants.

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[3] KLEBS, G.: op. cit., p. 8.

[4] E. g., those given by WHETHAM, W.C.D., Solution and Electrolysis, 215. Cambridge. 1895.

In making up culture media from the stock solutions of the constituent salts, it is necessary to dilute as far as possible before bringing the K_2HPO_4 and $Ca(NO_3)_2$ together. As was remarked by Klebs, this avoids, in a great measure, the separating out of quantities of $CaHPO_4$. This method is not completely satisfactory, however, and it would be better so to modify the proportions as to avoid the white precipitate entirely. I have planned to do this in future work. A solution made up of the quantities of the salts given by Knop dissolved in 93 parts of water is, of course, a 7 per cent. solution (since the iron is of such small amount it may be disregarded). This was further diluted to 1 per cent., 1.5 per cent., and 2 per cent. for growing the stock material.

Investigation.

At the outset it was found that if material in the palmella form were transferred from a 1 per cent., 1.5 per cent, or 2 per cent. solution where it had been growing for some time, to a solution of less than 0.5 per cent., the plants responded to the change in solution strength, and sent out long filaments from the original palmella masses (*figs. 7, 15, 16*). Also the palmella cells produced numerous zoospores. These germinated, as previously described, to produce, not the parent form, but the widely different filamentous one. Conversely, it was found that if filaments, produced as above, and growing healthily in a 0.2 per cent. or 0.25 per cent. nutrient solution, were changed to one of 1 per cent., 1.5 per cent., or 2 per cent. strength, the response was scarcely less well marked than in the other case; the cells of the filaments soon became spherical, and changed to the palmella form by dividing in both longitudinal and transverse planes (*figs. 4, 5*). In the strong solutions few or no zoospores were produced. Where a few were produced they changed, either directly or after the first or second division, to the palmella form.

With these two facts in view the attempt was made to determine where, in this change of solution strength, lies the necessary stimulus for the production of the corresponding change of form. A stimulus arising from a change of solution strength (always retaining the same salts in the same proportion) may be of any one of three different natures. (1) It may be of a chemical nature: *i. e.*, it may be due to a change in the amount of salts with which the organism is supplied. This implies three possibilities: (*a*) the plant may be affected by the increase or decrease in all the inorganic salts. (*b*) Again when such change is made it may be the change in amount of a single salt, say potassium nitrate, to which the plant responds. There are four possibilities under this head, one for each of the four salts used. (*c*) Again, it may be possible that neither of the preceding suppositions is true, but that the response is due, not to all the changes together, nor to any one alone, but to a combination of two or three of them. For example, it may be a change in the amount of potassium phosphate together with that of calcium nitrate, which acts as a stimulus. We know that, excepting in very weak solutions, these two salts react chemically upon each other, so that it is at least conceivable that an increase or decrease in both of them together might influence the plant differently from a corresponding change in only one. Under this head there are no less than ten possibilities, six taking the salts by twos, and four by threes. (2) Further, the stimulus may be of a physical nature and due, not to the change of salts at all, but to a change of osmotic pressure upon the living cell. This osmotic change may be effective in several ways. (*a*) It may be that the response is due to a change in the osmotic pressure of the solution in *general*. When the saline constituents of the solution are increased or decreased as a whole there is a corresponding change in the so-called osmotic strength. (*b*) However, it may be, as suggested by Copeland's recent work,[5] that the plant is more influenced by osmotic pressure when this is derived from one salt than by the same pressure derived from another. And if this be the case, then this influence is as complicated a one as that of the change in the conditions enumerated under (1) above. (3) Finally, the response in the plant may be due both to the stimuli from chemical composition and to those from osmotic pressure, a combination of (1) and (2) above.

My experiments were devised to determine primarily whether the stimulus is a chemical or a physical one. For this it is necessary to have solutions in which the relative and absolute amounts of saline constituents can be varied without changing the osmotic pressure of the salt content as a whole, and this must be brought about, as far as may be, without the introduction of any new conditions. It is necessary first to know the osmotic pressure of the complete Knop's solution. Now the pressure of any weak solution of several constituents is equal, at least approximately, to the sum of the partial pressures of the constituent salts, as these pressures would exist if the salts were separately put into a simple solution whose volume equaled that of the complex one. This principle makes it a simple matter to calculate the pressure of complex solutions of non-electrolytes. But the pressure of a simple solution of an electrolyte is not so easily calculated. On account of dissociation, such pressure is much greater than that of a solution of a non-electrolyte of the same gram-molecular concentration. It was this fact with which DeVries[6] was dealing when he derived his so-called isotonic coefficients. But since his range of concentration was very limited, he was unable to get at the truth of the matter; and the result is, that, though his coefficients may be, and doubtless are, true for a certain strength of solution, yet, since dissociation itself varies with the concentration, they are not true in general. However, in weak solutions dissociation becomes nearly complete, and if we assume that it is complete we shall probably be able to approach the truth much more nearly than by following the method of DeVries. My solutions are all very weak, and I have no doubt that my approximations by this method are, as a rule, quite nearly true. By assuming complete dissociation, the calculated osmotic pressure for any solution becomes that of an equimolecular solution of a non-electrolyte, multiplied by the number of ions derived from a molecule of the salt under consideration. This makes the calculation very simple. Taking the pressure of one gram-molecule per liter of some undissociated substance as a unit, *N*, table I gives the partial pressures of the complete solution which was used as standard. This is practically the same as a so-called 2 per cent. Knop's solution as usually made up. In the pressure columns, *a* is derived by assuming ionization complete; *b* by DeVries' method; and *c* gives the actual pressures for those salts for which data of dissociation could be found. The discrepancy between *a* and *b* lies almost wholly in the $Ca(NO_3)_2$. Here we should make a greater error in using DeVries' coefficients than in assuming complete dissociation. Column *n* gives the number of ions derived from a molecule, *i* is the ratio between the actual osmotic pressure and that of an equimolecular solution of a non-electrolyte. *N* at 0°C., is 1686^{cm} of mercury, or 22.18 atmospheres.

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[5] COPELAND, E. B.: The relation of nutrient salts to turgor. BOT. GAZ. **24**: 399. 1897.

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[6] DEVRIES, HUGO: Jahrbücher für wiss. Bot. **14**: 427. 1884.

DONDERS and HAMBURGER: Zeit. f. Physikal. Chemie **6**: 319. 1890.

TABLE I.

Salt	Gram-molecule per liter	n	Partial pressures in terms of N			
			a	b	c	i
$Ca(NO_3)_2$ - -	.07143	3	.21429	.28571	.175004	2.45
KNO_3 - - -	.02857	2	.05714	.08571	.055712	1.9
$MgSO_4$ - - -	.02381	2	.04762	.04762	.035712	1.5
K_2HPO_4 - -	.01664	4	.06655	.06736	....	....
Total pressure - - - -			.38559	.48641		

This 2 per cent. strength is the most concentrated solution I have used. Anything stronger kills the alga almost immediately. As we decrease the concentration below this standard 2 per cent. strength the dissociation becomes more and more complete. Thus as the concentration approaches zero as a limit the value of i approaches that of n. The value of i for the different concentrations used, as far as they can be calculated from the physical data at hand, are given in table II. Values for H_3HPO_4 are inserted for comparison, in place of those for K_2HPO_4. Table II also shows the total pressures of the various percentages in terms of *N*. The pressures in column *a* will be used throughout this paper, but instead of 0.3856 *N* I shall write 3856 $N \times 10^{-4}$, etc.

TABLE II.

Total pressures in terms of N.		"Per cent."	Values of i calculated from conductivity data				
a[7]	b		$Ca(NO_3)_2$	KNO_3	$MgSO_4$	K_2HPO_4	H_3PO_4
.38559	.486405	2	2.45	1.9	1.50	....	2.04
.289193	.364804	1.5	2.5 (?)	....	1.56	....	....
.192795	.243203	1	2.55	....	....	....	3.02
.096398	.121601	.5	2.66	1.92	....	....	....
.048199	.060801	.25	2.75(?)	....	1.68	....	....
.019279	.024320	.1	2.9	....	1.83	....	....
.00964	.013160	.05	3	2	1.89	....	4

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[7] The meaning of *a* and *b* is the same as in table I.

Having shown thus the partial and total pressures of the normal solution, I shall now describe the modifications of this solution which I have used. Four solutions were made up according to the formula given above, excepting that each was deficient in one of the four constituent salts. All of one salt, it was thought, should not be omitted, since then, as in higher plants, pathological responses would probably ensue. In these solutions the deficient salt was reduced to one tenth its normal quantity. The decrease in osmotic pressure, caused in each instance by this diminution of one salt, was calculated by the two methods just spoken of, and of each of the three other salts a sufficiency was added to increase the pressure by an amount equal to one third of the calculated decrease. Two full sets of these solutions were made up and used, one by assuming dissociation complete, the other by DeVries' method. These two solutions were identical in their effects upon the plant. Thus finally we have four solutions in each of which a deficiency of one salt is obtained, and the osmotic pressure of the solution is kept up to normal by an increase in the amounts of the other three salts. These amounts are not equally increased by weight, but according to the pressure which they produce when in solution. I shall designate these solutions as follows: A, deficient in $Ca(NO_3)_2$; B, deficient in KNO_3; C, deficient in $MgSO_4$; D, deficient in K_2HPO_4. K will denote the normal solution as given by Knop. The pressure will be expressed in terms of *N*.

The greater part of my work was done in a comparison of the effects upon the plants of the four solutions just described with the normal one of Knop. They were all diluted, for use in the cultures, to the various strengths given in table II. Also a number of tests were made with solutions of a single salt, using the four salts with which we have been dealing. These simple solutions were made up so as to have the same osmotic pressure as the complex ones just described. They will be designated as follows: E, a solution of $Ca(NO_3)_2$; F, of KNO_3; G, of $MgSO_4$; H, of K_2HPO_4. Calculations for these were made only by De Vries' coefficients.

In all, 278 cultures were made and record kept of each for five to fifty days. They were made at all seasons of the year and in two different years separated by a period of nine months, during which the material was kept alive and changes made from time to time but no records kept. It will be seen that there are two possibly very different questions to be answered in the problem before us. First, what are the conditions that bring about a change from the palmella form to the filamentous form; second, what are the conditions that bring about the reverse change? I shall divide the experimental data which are to follow into two groups corresponding to these two questions. Details of typical responses are given in the explanation of plates.

A. RESPONSES OF THE PALMELLA FORM.

In table III are given the results of fifty-five transfers of palmelloid cells into the different solutions. For convenience, all cultures having the same osmotic pressure are brought together. In the left hand column the letters denote the chemical content of the fluid, as just described. The number of cultures made is given in the second column. In the third is recorded the number of cultures in which the cells multiplied but remained of the palmella form after twenty to twenty-five days. Columns four and five (*Filaments*) give the number in which filamentous branches were produced from the original masses in five to twenty-five days. Those in column four produced few filaments, but continued mostly as palmella; those in column five produced many filaments. In columns six and seven (*Zoospores*) are the numbers which produced few or many zoospores within fifteen to twenty days. Column eight contains those which produced no zoospores for twenty-five days or more. The last two columns record cultures on which definite observatians are lacking, the first with regard to the production of zoospores, the second with regard to the production of filaments at the edges.

TABLE III.

Pressure 96 $N \times 10^{-4}$ or 16.19 cm. Hg.

Solution	Cultures	Palmella	Filam'ts		Zoospores				Filaments ?
			Few	Many	Many	Few	None	Doubtful	
K	12	1	2	4	10	2	..	..	5
A	5	..	1	4	5	..	..	..	..
B	5	..	2	3	3	2	..	..	..
C	5	..	..	5	5	..	..	..	..
D	5	2	..	3	5	..	..	..	..
E	8	..	2	3	7	1	..	..	3
F	2	..	2	..	2	..	..	..	..
G	2	..	1	1	2	..	..	..	..
H	2	..	1	1	2	..	..	..	..
Pressure 193 $N \times 10^{-4}$ or 32.37 cm. Hg.									
K	2	..	1	1	..	1	..	1	..
A	2	..	1	1	..	1	1	..	..
B	2	..	2	..	..	2	..	..	..
C	2	..	2	..	..	..	2	..	..
D	2	2	..	..	..	2	..	..	..
Pressure 482 $N \times 10^{-4}$ or 81.27 cm. Hg.									
K	2	..	..	..	2	..	..	..	2
Pressure 964 $N \times 10^{-4}$ or 161.86 cm. Hg.									
K	7	2	1	3	5	1	..	1	1
A	5	..	3	2	2	1	2	..	..
B	5	2	..	3	5	..	..	..	..
C	5	1	1	3	2	1	2	..	..
D	5	3	1	1	3	..	2	..	..
E	2	2	..	..	..	..	2	..	..
F	2	1	..	1	..	1	1	..	..
G	2	..	..	..	..	..	2	..	1

Pressure 1928 $N \times 10^{-4}$ or 323.71 cm. Hg.

Solution	Cultures	Palmella	Filam'ts		Zoospores			
			Few	Many	Many	Few	None	Doubtful
K	5	3	..	2	2	1	2	..
A	4	1	3	..	..	..	4	..
B	4	2	..	2	..	..	4	..
C	4	..	2	2	..	2	2	..
D	4	2	2	..	1	..	3	..
Pressure 2892 $N \times 10^{-4}$ or 487.59 cm. Hg.								
K	5	2	3	..	..	..	5	..
A	5	..	3	2	..	..	5	..
B	5	1	4	..	..	..	5	..
C	5	..	5	..	..	1	4	..
D	5	3	2	..	..	..	5	..
E	2	2	..	..	..	..	2	..
F	2	1	1	..	..	..	2	..
G	2	2	..	..	..	..	2	..
H	2	2	..	..	..	..	2	..
Pressure 3856 $N \times 10^{-4}$ or 647.42 cm. Hg.								
K	2	2	..	..	..	.	2	..
A	2	1	..	1	..	..	2	..
B	2	..	2	..	..	..	2	..
C	2	..	2	..	..	..	2	..
D	2	2	..	..	..	..	2	..

General Remarks on Table III.

The plant lived indefinitely in solutions K, A, B, C, and D, of all concentrations, and also in the lower concentrations of E, F, G, and H. But the higher pressures, when caused by the single salts, usually resulted in more or less speedy death. With a pressure of 2892 $N \times 10^{-4}$, it was quite a common thing to find the cells dead within a week. Sometimes they were perceptibly plasmolyzed before death ensued, sometimes not. Of the four simple solutions one appeared to affect the plant as much as another. The table shows the general result of the experiments almost graphically. In the higher concentrations the larger numbers appear mainly in the middle of the series, in the columns headed *Many*, while the third and eighth columns are nearly vacant. Passing to higher concentrations this gradually changes, until, in the highest, the reverse is true. The following conclusions may be drawn from these data.

1. *Nature of the stimulus.*—The effect of a solution of any given osmotic pressure upon the plant is both qualitatively and quantitatively the same, no matter what the chemical nature of the solution may be. If this effect were due to change in the amount of any one salt, then this fact would have appeared in the course of the experimentation, for my various solutions were devised to test this point. So we have eliminated, as far as the palmella form is concerned, the possibilities designated under 1, *a*, *b*, *c*, and 2, *b*, page 295. The stimulus cannot be of a chemical nature, nor can it be physical and depend more upon the presence of one salt than upon another. Also, we have eliminated 3 from among the possible primary stimuli. Considering the facts shown, it is impossible that the external stimulus should be either physical, with the necessary accompaniment of certain chemical factors, or yet chemical, with the necessary accompaniment of certain physical ones. It is, however, not only possible but probable that the external and primary physical stimulus may cause an internal and secondary chemical one. But that lies beyond the scope of the present research. Thus we are driven to accept 2, *a* as the truth in the matter, and to conclude that the response of the organism is determined by a physical, and never, as far as my experiments have gone, by a chemical stimulus. The effect of osmotic pressure upon the palmella form is a double one. I shall consider, first, the effect upon vegetative growth, and second, that upon reproduction.

2. *Response in vegetative growth.*—In my least concentrated solution (pressure $96N \times 10^{-4}$) the mode of cell division almost

invariably changed. In forty-six cultures only three are exceptions to this. The cells at the edge of the original mass elongate and become ellipsoidal or even cylindrical, and when division occurs it is by means of a wall lying transverse to the long axis of the cell (*figs. 7, 15, 16*). Thus filaments grow out around the periphery of the original group. The interior cells do not change their form and do not divide further; at length they form the center of a mass of long radiating filaments. Branching of a cell occurs by means of a papilla-like lateral outgrowth, which is cut off by a wall transverse to its long axis. The walls between the filament cells thus formed do not split; thus there is no bulging of the end walls and no separating of the cells. The older cells of a filament (for division occurs only at the tip) bulge at the sides and become barrel-shaped, but the end walls hold them fast together and prevent them forming true spheres (*fig. 25*). It is apparently this coherence of the walls and the orientation of the walls with reference to the form of the cell which constitutes the filamentous habit.

As the osmotic pressure of the fluid is increased there is a continual lessening tendency on the part of the plant to produce filaments, and there arise fewer and fewer filaments at the edge of the cluster. Also these filaments tend to go back, by a rounding and breaking apart of their cells, into the original form. The older cells of the filaments do this, while the younger ones continue to advance by cylindrical cells (*figs. 4, 5, 10, 25, 31, 34*). In my most concentrated solution (pressure $3856 N \times 10^{-4}$) there were still five cultures out of ten wherein filaments were formed, but in all but one of these the filaments were only two or three cells in length. Back of these cells a thick strand of palmelloid cells marked the path of the advancing filament tip (*figs. 20, 30*). This change of the cylindrical cell to a spherical one, accompanied, as it is, by a corresponding change in the direction of new walls, will be discussed when we consider the responses in filaments. For the present it is enough to note that with increasing pressure the tendency to produce peripheral filaments decreases, both in the number and in the length they attain before reverting to the palmella form. In my weakest solution they often attained a length of twenty-five to thirty cells (but the older cells here are destroyed in the act of producing zoospores, which will presently be discussed), while in my strongest solution the few filaments produced seldom attained a length of over two cells. The tips of filaments are quite sharply pointed in weak solutions, but blunt in strong ones.

A maximum pressure beyond which filament growth cannot take place lies somewhere between $1928N\times10^{-4}$ and $2892N\times10^{-4}$. No limit was found beyond which no filaments at all could be formed.

3. *Response in reproduction.*—The most marked response of this form to the change in osmotic pressure conditions is the sudden production of enormous numbers of zoospores. In some of my cultures almost every original cell has thus emptied out its contents within ten or twelve days of the date of transference. In general, within a fortnight and often within five days these very weak solutions become literally swarming with zoospores, and their production continues as long as the pressure remains low. Zoospores come both from original spherical or parenchyma-like cells and from the older cells of filaments radiating from the palmella mass. This method of producing zoospores has often been resorted to by various writers, and Klebs especially has found it successful in a great variety of forms. But, as far as I know, previous writers have not tried to determine the real nature of this weak solution stimulus.

As would be expected from the response in vegetative growth, when zoospores come to rest in these weak solutions and begin to enlarge, they do so by elongating into a cylinder, which soon divides transversely and later produces branches (*figs. 8, 14, 18, 32*). Thus the response to low osmotic pressure of a zoospore which has come to rest is the same as that of a young cell of the palmella form. Zoospores often grow at both ends, however, which I have not observed in case of other cells. The two cells formed by the first division of the zoospores thus sometimes become a base from which reach out two long branching filaments (*figs. 2, 18*).

In solutions of greater pressure zoospores are produced more and more tardily and in fewer and fewer numbers. The limit to the healthy production of zoospores lies at a pressure of about 964 $N \times 10^{-4}$. Their production in a higher pressure is exceptional and I have never seen them produced at all in my strongest solution. With a pressure of 964 $N \times 10^{-4}$ all zoospores germinate very soon in the manner already described; some of them go directly into the palmella form while most of them germinate to produce filaments of two to five cells, and then these pass into the palmella condition by rounding and breaking up (*figs. 17, 19*). Thus it often comes about that cultures with this pressure show both forms growing together, short young filaments and free round cells as well as parenchyma-like masses. With pressures above the observed limit the spores, when they are produced, usually grow directly into the palmella form, and then continue to grow slowly, or go into the resting stage. I have given little attention to the fate of these resting spores. They retain their green color and remain indefinitely at the bottom of the culture dish. Klebs has described these and states that they may be made to germinate after complete drying out.

B. RESPONSES OF THE FILAMENTOUS FORM.

Experimental data from 169 transfers of filaments into the various solutions are presented in table IV, which is constructed on the same plan as table III. Whether or not the original material changed into the palmella form is shown in the third, fourth, and fifth columns. Cultures in which all or nearly all the cells went over into the other form within a period of thirty days are recorded in the third column; those in which some, but not all, went over, and long filaments persisted after twenty to forty days, are indicated in the fourth column. The fifth column records cultures in which the original filaments did not change in form but continued as such for twenty to forty days. The results as to the production of zoospores, whether many, few, or none, are tabulated under those respective heads. Where zoospores were produced, but many or all of them did not germinate, the numbers are placed in the column headed *Ungerm.*

TABLE IV.

Pressure 96 $N \times 10^{-4}$ or 16.19 cm. Hg.

Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None	
K	6	..	..	6	6	..	..	..	..
A	6	..	..	6	6	..	..	..	..
B	6	..	..	6	6	..	..	..	..
C	6	..	..	5	5	..	2	..	1
D	6	..	..	5	5	..	..	..	1
E	7	..	1	6	5	2	..	..	1
F	1	..	..	1	1	..	..	..	..
G	1	..	..	1	1	..	..	..	..
H	1	..	..	1	1	..	..	..	..

Pressure 193 $N \times 10^{-4}$ or 32.37 cm. Hg.

Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None	
K	3	..	..	3	3	..	3	..	..
A	3	..	3	..	2	1	2	..	..
B	3	..	..	3	3	..	3	..	..
C	3	..	..	3	3	..	3	..	..
D	3	..	..	3	3	..	..	..	..
E	2	..	..	2	2	..	..	..	..

Pressure 482 $N \times 10^{-4}$ or 81.27 cm. Hg.

Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None	
K	4	..	1	3	..	..	3	..	..
A	3	3	..	..	3	..	3	..	..
B	3	3	..	..	3	..	3	..	..
C	3	3	..	..	3	..	3	..	..
D	3	..	3	..	3	..	3	..	..
E	3	..	2	1	1	..	2	..	..

Pressure 964 $N \times 10^{-4}$ or 161.86 cm. Hg.

Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None	
K	7	3	1	2	7	..	6	..	..
A	6	1	4	1	6	..	3	..	..
B	6	4	..	2	6	..	3	..	..
C	6	3	3	..	3	3	3	..	..
D	6	2	3	1	6	..	3	..	..
E	6	..	4	2	2	3	2	1	2
F	1	..	1	..	..	..	..	1	..
G	1	..	1	..	..	1	1	..	..
H	1	..	1	..	1	..	..	..	..

Pressure 1928 $N \times 10^{-4}$ or 323.71 cm. Hg.

Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None	
K	6	3	2	1	2	4	4	..	..
A	5	3	1	1	1	2	1	2	..
B	5	5	..	..	2	..	2	3	..
C	5	5	..	..	2	3	3	..	..
D	5	3	..	..	..	3	1	..	2
E	5	..	2	..	..	..	..	2	3

Pressure 2892 $N \times 10^{-4}$ or 487.59 cm. Hg.

Solution	Cultures	Palmella		Filaments	Spores				Died
		All	Few		Many	Few	Ungerm.	None	
K	1	1	..	..	..	..	..	1	..
A	3	2	1	..	..	1	1	2	..
B	3	3	..	..	..	2	..	1	..
C	3	2	1	..	..	2	2	1	..
D	3	2	1	..	..	..	..	3	..
E	6	1	..	..	..	..	..	2	5
F	1	..	1	..	..	..	..	1	1
G	1	..	..	..	..	..	..	..	1
H	1	..	..	..	..	..	..	..	1

General Remarks on Table IV.

The only solutions in which death regularly ensued were the more concentrated ones made from single salts; *i e.*, E, F, G, H. But in almost every case where the original filaments died they evinced a great tendency to change into the palmella form before death ensued. Often they did not die until they had actually taken the palmella form. Where a definite response was not observed before death, the cultures are recorded in the column headed *Died*. The graphic arrangement of the numbers in this table is even more striking than that in table III. The conclusions to be drawn from the above set of results will be given in the same order as were those derived from the experiments upon the palmella form.

1. *Nature of the stimulus.*—The stimulus with which we have to deal is always of an osmotic nature. Indeed, this generalization would have been expected from the nature of the stimulus affecting palmelloid cells, and from the data which we are now considering the same facts can be cited in its support.

2. *Response in vegetative growth.*—Filaments placed in the weakest solution used (pressure $96\, N \times 10^{-4}$) continued indefinitely to grow without change in form. No going over to palmella was observed until time enough had elapsed for great concentration of the solution through evaporation. The rule is, if filaments are left in a dish without renewal of fluid, they change to the other form at the end of a period of thirty to fifty days. By this time the culture is often nearly dry. Also, by addition of water from time to time filaments may be kept growing in a dish as long as one chooses. So there is little room for question as to what the nature of the stimulus producing this very tardy response really is. With higher osmotic pressures the ability of the alga to continue as filaments rapidly decreases, the first indication of such an effect being the blunting of the terminal cells. A limiting pressure for free filament growth lies apparently somewhere in the region between the pressure of $193\, N \times 10^{-4}$ and $482\, N \times 10^{-4}$. With a pressure of $964\, N \times 10^{-4}$ only eight out of forty cultures showed good growing filaments after thirty days, while many of these cultures had lost all vestige of them within that time. Not a single one of twenty-two cultures having a pressure of $2892\, N \times 10^{-4}$ showed typical filaments at the end of such a period, and there were only four which showed filaments at all. As in the other form,

an absolute limit to the production of filaments was not found. A comparison of the limit found here with that found in the case of palmella material will be made later. Filaments floating on a strong solution change to the palmella form much more slowly than those at the bottom of the dish.

The morphological details of the response just discussed are as follows. If a filament be placed in a strong solution the oldest cells are the first to be visibly affected. They continue rounding up beyond the barrel-shaped stage and become nearly or quite spherical. This process is accompanied necessarily by a splitting of the common walls from their margins inward, until, when the cells have attained the spherical form, they are practically free from one another (*figs. 4, 5*). They may not break entirely apart, however, but may remain clinging together in loose strands. The limitation upon the direction of new walls (*cf.* discussion of the responses of palmella form, *p. 300*) is now apparently removed, and the cells proceed to divide in all directions. The new cells may separate entirely (*fig. 10*), may remain loosely attached to one another (*fig. 34*), or may form an irregular parenchyma-like colony (*figs. 6, 11*). In the latter case the rounding is of course incomplete, but such cells are always nearly isodiametric. Cell division goes on much more slowly in the palmella form than in the filamentous.

3. *Response in reproduction.*—The response is the same here as in the other forms and is equally marked. The pressure limit to the production of zoospores by the filamentous form is somewhat above 964 $N \times 10^{-4}$. The limit to their germination into long filaments is below 482 $N \times 10^{-4}$. Zoospores are produced here in the way already described. The length of a filament, even in a weak solution, is limited by the fact that its oldest cells are continually becoming zoosporangia and thus losing their contents (*fig. 16*).

General considerations.

The question which this research was undertaken to answer has been answered very clearly as far as the answer goes. The stimulus to which the alga responds by a change of form is a change in the osmotic pressure of the surrounding fluid. This must be accompanied by a corresponding change in the relation of water to the protoplasm. Whether or not extraction of water brought about by solutions of non-electrolytes or by evaporation from a moist substratum will be accompanied by the same response as that brought about by a solution of electrolytes, my experimentation has not yet gone far enough to show. It is worthy of remark, however, that when found wild this alga was in the palmella form, and was growing in air on moist bark. Loeb[8] has recently brought about artificial parthenogenesis, both in the eggs of echinoderms and annelids by placing them in a solution which would extract water from them osmotically. The result was the same whether electrolytes or non-electrolytes were used. Thus in these eggs the phenomena of segmentation and growth are profoundly influenced by osmotic pressure. Whether or not his results have any connection with those given in the present paper is an open question. The recent work upon various grains and forage plants by Buffum and Slosson[9] furnishes data which may have a somewhat closer connection with my own. Absorption by seeds, their germination, and the growth of the plant were all greatly retarded in a strong solution and accelerated in a weak one. And this was true without regard to the chemical nature of the dissolved substance; a number of electrolytes were used and also non-electrolytes. On the other hand, my experiments have not been at all in accord with those of Copeland (*loc. cit.*) when he found, with peas, maize, and buckwheat, that K-ions are osmotically more potent than any other ions in his nutrient solutions. Some erratic cultures in my series seem to support his statements, but no generalization can be made from them. Of interest, also, in this connection are the recent researches of Yasuda.[10] Working with infusoria, he finds that these organisms are able to adjust themselves to solutions of quite high concentration, and that, in general, the limit to their power of adjustment seems to be osmotically about the same, no matter what salts are used. In other words, the limit is apparently one of osmotic pressure. It is probable that many of the so-called chemical or nutritive effects of dissolved substances upon the plant organism may turn out, upon further investigation, to be wholly or in part osmotic.

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[8] LOEB, J.: Further experiments on artificial parthenogenesis and the nature of the process of fertilization. Amer. Jour. Physiol. **4**: 178. 1900.

———: Artificial parthenogenesis in annelids (Chætopterus). Science N. S. **12**: 170. 1900.

[9] (1) SLOSSON, E. E., and BUFFUM, B. C.: Alkali studies II, Bulletin 39, Wyoming Exp. Sta. 1898.

(2) BUFFUM, B. C.: Alkali studies III, Ninth Ann. Report, Wyoming Agric. Exp. Sta. 1899.

(3) SLOSSON, E. E.: Alkali studies IV, same. 1899.

(4) BUFFUM, B. C., and SLOSSON, E. E.: Alkali studies V, Tenth Ann. Report, Wyoming Agric. Exp. Sta. 1900.

In a weak solution vegetative growth is very much more rapid than in a strong one This may be due to the fact that in a strong solution the water content of the protoplasm is reduced in amount below the limit for optimum lability. When the plant grows fastest and best, it is in the filamentous form. In the weak solution, where activity seems to be at a maximum, the ions of the electrolytes, which are essential for metabolism, are not plentiful. This may suggest how the cylindrical form of cell with its increased surface[11] may be advantageous. At any rate, we may be sure that the greater surface of the cylinder puts the plant into better condition for exchange of material with its surrounding medium. On the other hand, the more concentrated solution not only withholds water from the cells, but presents a demand upon them for water. The cell meets this in part by offering as small a surface as possible to the solution. In this case, although the requisite ions may be present, and even in the right number, the scarcity of water in the

[10] YASUDA, ATSUSHI: Studien über die Anpassungsfähigkeit einiger Infusorien an concentrirte Lösungen. Jour. Coll. Sci. Imp. Univ. Tokyo **13**: 101–140. 1900.

[11] In a cylinder, the lateral surface is greater than that of a sphere of the same volume, as long as the ratio of the length to the diameter equals or exceeds 2.727. In typical filament cells of this alga the ratio of the diameters is 3, and it is often 4 and even greater. It is seldom less than 2.8. Thus it is shown that the filament cell offers more surface to the surrounding medium through its lateral walls alone, than does the palmella cell of equal volume.

protoplasm may so decrease the lability that rapid growth is impossible.

What may be the mechanics of this rounding up of cylindrical cells when placed in a concentrated solution is one of the most important problems suggested by the present research. The fact that the dead cellulose membrane is almost entirely reshaped during this process, without being dissolved, renders it probable that the change in form is directly caused by some turgor change within the cell. In a rounding cell the membrane moves and changes its form and, since it is entirely inert, the source of this motion must be either in the activity of the protoplasmic body itself, or it must be in the turgor pressure of the mass of liquid within. But since protoplasm and cellulose wall can be parted so readily during plasmolysis, the first alternative is well-nigh untenable. If the wall be forced into the spherical shape by a change in the pressure from within, this must be brought about by a change in the mass of the contained liquids. Now, this slight change in mass which might produce a change in the turgor of the cell is most probably due to an alteration in the amount of cell sap within the vacuole. When the surrounding medium suffers change in concentration, a change in the volume of the vacuole may come about through the protoplasmic sac, either secreting liquid or acting merely as a semipermeable membrane.

When filaments are placed in a concentrated solution their behavior suggests at once partial plasmolysis. Water may be extracted, the turgor pressure on the walls may be decreased, and by the forces of surface tension and cohesion the protoplasm may tend to round itself up into a sphere. If this be true, we have an explanation of the lateral bulging which accompanies the longitudinal shrinking of the cellulose envelope. If the protoplasm tended to assume a spherical form within the cylindrical wall, the pressure upon this would be decreased first at the angles. At the same time it would be relatively increased upon the lateral walls near their middle. Thus would come about a bulging of the lateral walls outward, and hence a shortening of the cell and a drawing of the end walls towards each other. But the internal pressure is to be counted as almost nothing at the angles, while it is still considerable in the middle of each end wall. So the margins of the end walls would approach the middle of the cell more rapidly than do their central portions, and splitting of the common membrane of two adjacent cells would necessarily ensue. Several facts were observed in the cultures which seem to support some such hypothesis as the one just stated. I have placed filaments in a solution where they were completely plasmolyzed and killed without any change in form. In solutions a little less concentrated they are not plasmolyzed but round up rapidly and soon die, often in the palmella condition. With a still lower pressure the filament cells round up more slowly and live. Another fact suggesting this idea is that floating filaments can resist a stronger solution, and can resist it longer, than sunken ones. The former are to some extent in contact with the air, and thus present less surface than the latter to the liquid. Still another observation bearing upon this hypothesis of partial plasmolysis is that cylindrical cells are the only ones which are able to change their form after they have become mature. A spherical cell must remain so till it divides, even if it be in a solution of very low pressure. Only two other observations with which I am acquainted bear upon this question: Yasuda (*loc. cit.*) says infusoria in concentrated solutions tend to approach the spherical form, and Klebs (*loc. cit.*) notes that the form of *Stigeoclonium tenue* with which he was working had a tendency to produce round cells in a concentrated solution. The very marked response in the orientation of the planes in segmentation may be traceable to the change in the form of the cell, or it may not. The observed fact is that vegetative cells under low osmotic pressure divide only across their long axis (across the axis of a lateral outgrowth in the case of a branch), while under the influence of high osmotic pressures the cells divide in all directions. It is remarkable, too, that the stimulus determining the position of walls in vegetative division also brings about the extreme segmentation which occurs in the zoosporangium. Perhaps this fact is attributable to the increased general activity in weak solutions. It has no relation to the form of the cell, since zoospores are produced from both spherical and cylindrical cells, as well as from those of intermediate shape.

My approximations to the pressure limits for the different responses are given in table V. The numbers are all coefficients of $N \times 10^{-4}$.

TABLE V.

	Maximum limit to free production of filaments	Maximum limit to production of any long filaments	Minimum limit to free production of palmella form	Minimum limit to production of any palmelloid cells	Maximum limit to free production of zoospores	Maximum limit to any production of zoospores	Maximum limit to free germination of zoospores
From palmella	1928	2892	2892	964	964	1928	964
From filaments	193	482	1928	482	1928	2892	193

From table V it will be observed that with the exception of the limits for zoospore production, all are much higher for the palmella form than for the filamentous. Perhaps the palmella cells become attuned to conditions of high pressure, so that after they have existed under these conditions for some time they are enabled to form filaments, and maintain them, at a higher pressure than was possible previously. Buffum and Slosson (*loc. cit.*, 3) state that there was a continual increase in the salt content of seeds which were imbibing water from a salt solution. Thus the difference between internal and external pressures in the case of seeds is being gradually decreased, and this means that the effect of the external pressure in retarding absorption of water is gradually diminishing also. Now if this be true in these algal cells, we may have in it an explanation of the fact that the palmella form (always growing in concentrated solutions) becomes to a greater or less extent attuned to the effects of high pressure; the ratio of internal to external pressure may be nearer unity for a palmelloid cell than for a filamentous one. That the nature of the protoplasm itself is changed by prolonged subjection to the action of a concentrated solution is indicated by a comparison of the maximum concentrations for zoospore germination in the two forms. Zoospores from a palmelloid cell are able to produce and maintain filaments at a much higher pressure than can those produced from a filament. The two kinds of zoospores are physically alike, but are physiologically very different. It needs to be said here that in cases where the tables record ungerminated zoospores there are also many which did germinate to form filaments of several cells. In cultures so marked, however, the majority of zoospores failed to germinate in that way.

Summary.

My results are as follows:

1. The responses of *Stigeoclonium* (*tenue* ?), both in form and in reproductive activity, which accompany a change in concentration of the Knop's solution in which it is growing, are due to changes in the osmotic pressure of the medium, and are in no way functions of its chemical composition.

2. A high osmotic pressure affects the plant in four ways: (*a*) it decreases vegetative activity; (*b*) it inhibits the production of zoospores; (*c*) it causes cylindrical cells to become spherical; (*d*) it frees the alga from certain limitations as to the orientation of planes of cell division.

3. A low pressure has the following effects: (*a*) it increases vegetative activity; (*b*) it accelerates the production of zoospores; (*c*) it causes forming cells to become cylindrical; (*d*) it determines the orientation of the planes of cell division.

4. A zoospore which has come to rest responds in the same way as a palmelloid cell.

5. Cells of the palmella form become slightly attuned to the high external pressures of concentrated solutions and exhibit some responses quantitatively different from those of filaments.

In closing I wish to express my thanks to Professor F. C. Newcombe, of the University of Michigan, for much valuable suggestion and advice in the inception of the work, and to

Professor C. R. Barnes, of the University of Chicago, for aid in its completion. Also, I take occasion here to acknowledge with thanks much kind assistance regarding physical chemistry rendered me by Dr. K. E. Guthe, of the University of Michigan.

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EXPLANATION OF PLATES XVII AND XVIII.

All of the figures are reproductions of photomicrographs taken with a Zeiss camera and Zeiss microscope. The first five are magnified about 450 diameters, the rest only about 140. All but *figs. 1–5* were taken as the plants lay in or upon the nutrient medium in the culture dish. The others were made from slide mounts in water.

PLATE XVII.

FIG. 1. Typical palmella form. *a*. Vegetative cell, showing clear area not covered by chloroplast. *b*. The same, but preparing to produce zoospores. *c*. Cells which have divided, but whose daughter cells are not yet separated.

FIG. 2. Typical filaments.

FIG. 3. Zoospores, immediately after they have come to rest. The clear area is the part of the periphery not bounded by the chloroplast.

FIGS. 4 and 5. Typical filaments changing to the palmella form by rounding and separation of cells.

FIG. 6. Culture no. 50. Exposure May 10. Typical palmella material. The mass at the right shows a parenchymatous colony. This was used for original material in many of the following cultures.

FIG. 7. Culture no. 144*a*. Palmella from no. 50 put into K, $96 N \times 10^{-4}$ May 4, 1900. Exposure May 28. Original palmella masses with long filaments at periphery. Also young filaments from zoospores. The original masses are out of focus.

FIG 8. Culture no. 147*a*. Palmella from no. 50 put into C, $96 N \times 10^{-4}$ May 7, 1900. Exposure May 28. Original masses have grown out into long filaments whose older cells have rounded. Many young filaments from zoospores.

FIG. 9. Culture no. 132*a*. Palmella from no. 50 put into K, $1928 N \times 10^{-4}$ May 7, 1900. On May 23 many new filaments had been produced from zoospores but were rapidly breaking up. Exposure June 12. None of the original material is shown, the new filaments are almost entirely palmella. Compare this with *fig. 12*, which is from the same culture, exposure on the same day, but shows the original material. It has multiplied much, and one filament is shown reaching out from the edge of the old colony.

FIG. 10. Culture no. 141*b*. Palmella from no. 50 put into C, $964 N \times$

10^{-4} May 7, 1900. Exposure June 12. Filaments have grown from the original palmelloid cells but these break up as soon as the cells become a few days old. Original material is seen at the upper left.

FIG. 11. Culture no. 126*a*. Palmella from no. 50 put into K, 2892 $N \times 10^{-4}$, May 7, 1900. Exposure May 28. Typical palmella form.

FIG. 12. See explanation of *fig. 9*.

FIG. 13. Culture no. 128*a*. Palmella from no. 50 put into B, 2892 $N \times 10^{-4}$ May 7, 1900. Exposure May 28. Typical palmella form. Material moved slightly during exposure, but its form is well shown.

FIGS. 14 and 15. Cultures no. 145*a* and 145*b*. Both these cultures palmella from no. 50 put into A, 96 $N \times 10^{-4}$ May 7, 1900. Exposure May 28. Filaments radiate from the original colonies and many new filaments have been formed from zoospores. In *fig. 14* the material was floating, in *fig. 15* it was resting on the bottom of the dish. The latter shows no filaments from zoospores. They usually float on the surface.

FIG. 16. Culture no. 149*b*. Palmella from no. 50 put into E, 76 $N \times 10^{-4}$ May 7, 1900. May 23 many new filaments had been formed both at edges of original masses and by zoospores. Exposure May 28. At that time the filaments from zoospores were long and their older cells were rounding up. These are shown. Zoospores and young filaments were still plentiful but did not occur in the field where the exposure was made. Many of the older cells are empty, having produced zoospores.

FIG. 17. Culture no. 139*a*. Palmella from no. 50, put into A, 964 $N \times 10^{-4}$ May 7, 1900. Exposure June 16. Zoospoores have been formed in large numbers and many have germinated, but they break apart and form palmelloid cells almost immediately. There are no filaments here more than three or four cells in length. Some resting spores which will not germinate are shown.

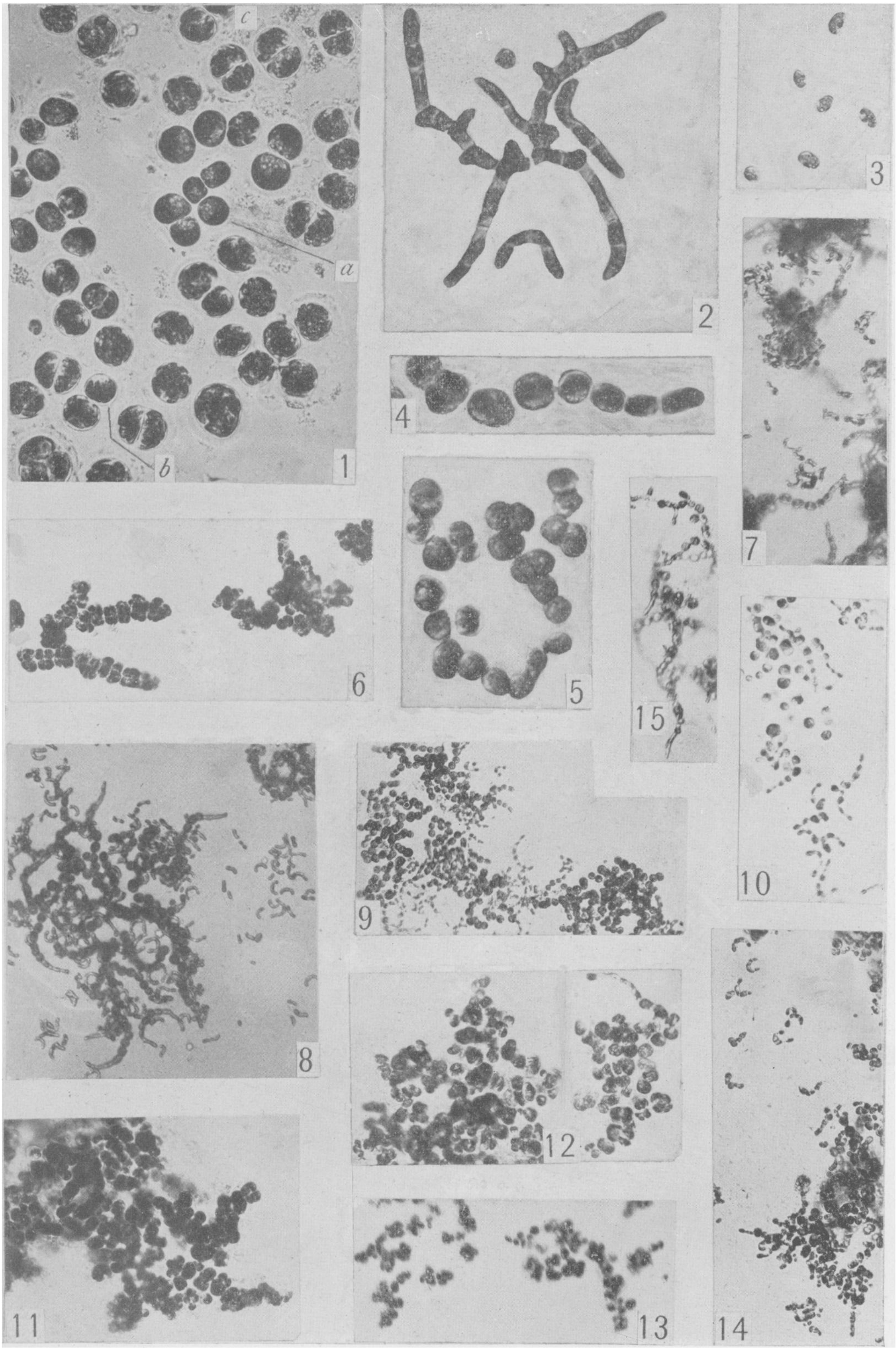
Fig. 18. Culture no. 148*a*. Palmella from no. 50, put into D, 96 $N \times 10^{-4}$ May 7, 1900. Exposure May 26. Shows typical weak solution response. Surface covered with filaments produced from zoospores.

Fig. 19. Culture no. 140*a*. Palmella from no. 50, put into B, 964 $N \times 10^{-4}$ May 7, 1900. Exposure June 15. Many zoospores were produced and germinated but went almost immediately into the palmella form. Figure shows filaments, palmella and some germinating spores.

FIG. 20. Culture no. 130*a*. Palmella from no. 50, put into D, 2892 $N \times 10^{-4}$ May 7, 1900. Exposure May 28. Palmella still with scarcely any tendency to form filaments at edges.

FIG. 21. Culture no. 125*a*. Palmella from no. 50, put into E, 96 $N \times 10^{-4}$ May 8, 1900. Exposure June 15. Long filaments have been produced from zoospores. Some of their older cells are becoming rounded. Many zoospores not yet germinated are seen. Most of these germinated later.

FIG. 22. Culture no. 108*a*. Filaments from no. 83*a* (whose appearance



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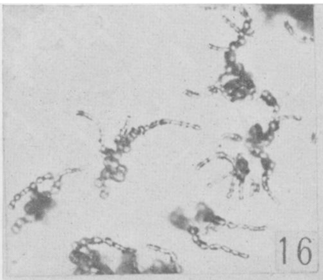
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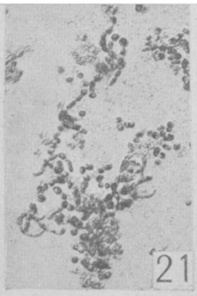
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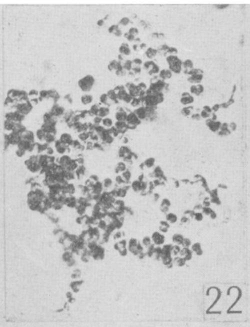
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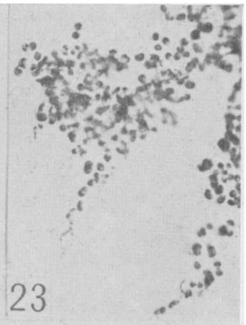
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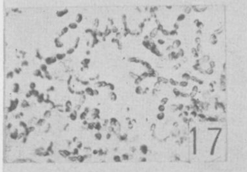
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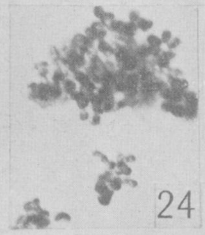
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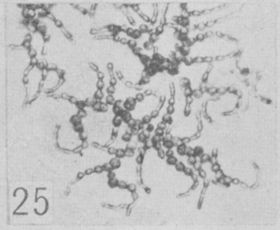
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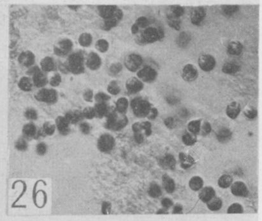
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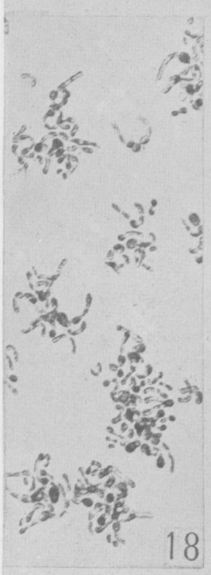
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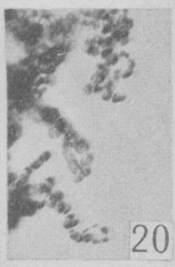
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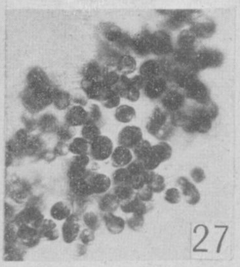
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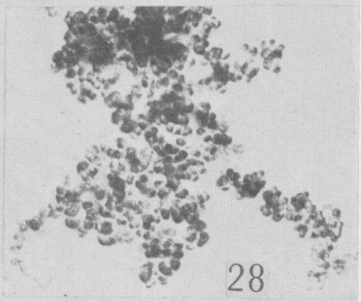
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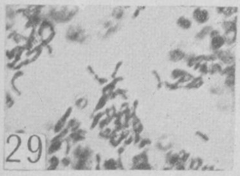
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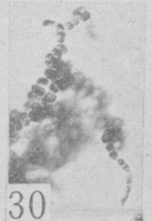
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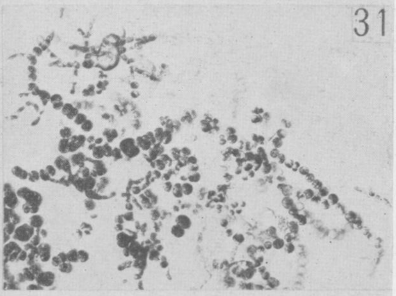
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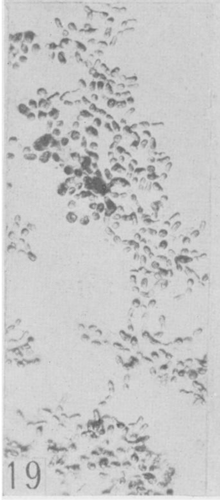
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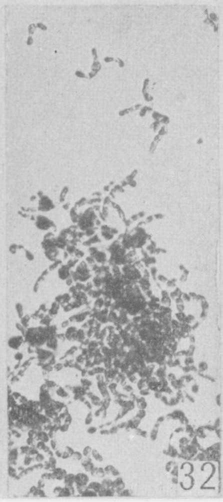
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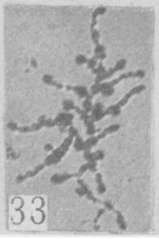
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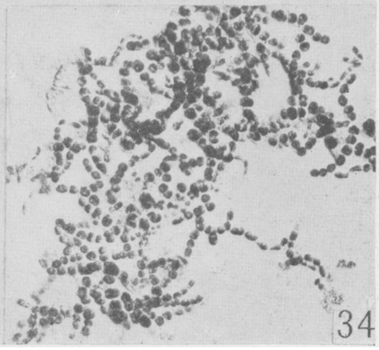
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was exactly that shown in *fig. 18*), put into K, $1928\ N \times 10^{-4}$ May 8, 1900. Exposure June 15. Very few and very short filaments at the edges of large palmella masses.

FIG. 23. Culture no. 109. Filaments from no. 83*a*, put into A, $1928\ N \times 10^{-4}$ May 8, 1900. Exposure June 15. Very few filaments at the edges of large palmella colonies.

FIG. 24. Culture no. 105*a*. Filaments from no. 83*a*, put into C, $2892\ N \times 10^{-4}$ May 8, 1900. Exposure May 28. Palmella with scarcely any tendency to form filaments at edges.

FIG. 25. Culture no. 118*b*. Filaments from no. 83a, put into D, $964\ N \times 10^{-4}$ May 8, 1900. Exposure June 15, Filaments were produced from zoospores and have grown long. Their older cells have rounded and the very oldest ones are truly palmella.

FIG. 26. Culture no. 176*a*. Filaments from no. 148*a* (*fig. 18*) put into K, $2892\ N \times 10^{-4}$ May 25, 1900. Exposure June 15. Nothing but palmella.

FIG. 27. Culture no. 111*b*. Filaments from no. 83*a*, put into C, $1928\ N \times 10^{-4}$ May 8, 1900. Exposure June 15. Nothing but palmella.

FIG. 28. Culture no. 116*a*. Filaments from no. 83*a*, put into B, $964\ N \times 10^{-4}$ May 8, 1900. Exposure June 15. Very few filaments at periphery of irregular masses of palmella.

FIG. 29. Culture no. 169*a*. Filaments from No. 148*a*, put into C, $964\ N \times 10^{-4}$ May 25, 1900. Exposure June 15. A mixed culture, showing palmella, young and old filaments, the latter changing to palmella, and zoospores. The figure is poor. True palmella is shown at the upper right.

FIG. 30. Culture no. 106*a*. Filaments from no. 83*a*, put into D, $2892\ N \times 10^{-4}$ May 8, 1900. Exposure May 28. Original filaments are traced by knotted strands of palmella. At the tip of each strand are two or three filament cells.

FIG. 31. Culture no. 123*a*. Filaments from no. 83*a*, put into C, $96\ N \times 10^{-4}$ May 8, 1900. Exposure June 15. Many new filaments were produced from zoospores. The original material has, in good part, gone to palmella by increased concentration from evaporation. The younger filaments continue filaments still, but their older cells become spherical.

FIG. 32. Culture no. 120*a*. Filaments from no. 83*a*, put into K, $96\ N \times 10^{-4}$ May 8, 1900. Exposure May 28. Original material growing as filaments. No true palmella. Also many young filaments and zoospores.

FIG. 33. Culture no. 122*a*. Filaments from no. 83*a*, put into B, $96\ N \times 10^{-4}$ May 8, 1900. Exposure May 28. Original material still growing finely as filaments. Young filaments and zoospores.

FIG 34. Culture no. 158*a*. Filaments from No. 148*a*, put into H, $964\ N \times 10^{-4}$ May 25, 1900. Exposure June 15. There are scarcely any good filament cells. The palmella cells lie in loose rows, marking the direction of the original filaments.